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M FARRUKH SALEEM
BASHARAT ALI
MUHAMMAD HAMZAH SALEEM
MUHAMMAD RIZWAN

See next page for additional authors

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SARWAR, MUHAMMAD; SALEEM, M FARRUKH; ALI, BASHARAT; SALEEM, MUHAMMAD HAMZAH; RIZWAN, MUHAMMAD; USMAN, KAMAL; KEBLAWY, ALI EL; ALI, ASJAD; AFZAL, MUHAMMAD; SHETEIWY, MOHAMED S.; and ALI, SHAFAQAT (2022) "Application of potassium, zinc and boron as potential plant growth modulators in Gossypium hirsutum L. under heat stress," Turkish Journal of Agriculture and Forestry; Vol. 46: No. 4, Article 13. https://doi.org/10.55730/1300-011X.3026
Available at: https://journals.tubitak.gov.tr/agriculture/vol46/iss4/13

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This article is available in Turkish Journal of Agriculture and Forestry: https://journals.tubitak.gov.tr/agriculture/vol46/iss4/13
Application of potassium, zinc and boron as potential plant growth modulators in *Gossypium hirsutum* L. under heat stress

Muhammad SARWAR\(^1\), Muhammad Farrukh SALEEM\(^1\), Basharat ALI\(^{1,2,*}\), Muhammad Hamzah SALEEM\(^3,*\),
Muhammad RIZWAN\(^5\), Kamal USMAN\(^6\), Ali EL-KEBLAWY\(^7\), Asjad ALI\(^8\), Muhammad AFZAL\(^9\),
Mohamed S. SHETEIWY\(^8\), Shafaqat ALI\(^{3,10,*}\)

\(^1\)Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
\(^2\)Department of Agricultural Engineering, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan
\(^3\)Office of Research, Office of VP for Research & Graduate Studies, Qatar University, Doha, Qatar
\(^4\)Agricultural Research Station (ARS), Office of VP for Research and Graduate Studies, Qatar University, Doha, Qatar
\(^5\)Department of Applied Biology, Faculty of Sciences, University of Sharjah, Sharjah, United Arab Emirates
\(^6\)Department of Agricultural Sciences, The University of Lahore, Lahore, Pakistan
\(^7\)Department of Agricultural Sciences, Government College University, Faisalabad, Pakistan
\(^8\)Department of Agronomy, Faculty of Agriculture, Mansoura University, Mansoura, Egypt
\(^9\)Department of Biological Sciences and Technology, China Medical University, Taichung, Taiwan

**Abstract:** High temperature stress at reproductive stages of cotton crop severely affects the yield and quality of cotton crop under changing climatic conditions. To alleviate the adverse effects of high temperature stress on cotton crop, the regulatory effects of potassium (K), zinc (Zn), and boron (B) were assessed by applying different temperature regimes at three reproductive stages of cotton crop under field and glasshouse conditions. Cotton plants were subjected to low (32/20 °C ± 2), medium (38/24 °C ± 2), and high (45/30 °C ± 2) temperatures under glasshouse, but sown at specific dates in field to provide different temperatures at three reproductive stages. High-temperature stress at squaring, flowering and boll formation stages in both field studies increased relative cell injury (RCI), total soluble proteins (TSP), reactive oxygen species and reduced fiber yield attributes i.e. total number of bolls per plant (TNBPP), number of sympodial branches per plant (NSBPP), and quality traits. For example, RCI, TNBPP and fiber fineness were reduced by 73%, 42% and 29%, respectively under supra thermal regime (SupTR) of glass house study over the optimal thermal regime (OpTR). Foliar application of K and Zn followed by B increased TSP, RWC, TNBPP, NSBPP, fiber fineness, fiber length and fiber strength. Further, foliar spray of K and Zn followed by B also reduced H\(_2\)O\(_2\) under SupTR and SubTR over the OpTR. The findings of the present study clearly demonstrate that foliar spray of Zn, K and B alleviated adverse effects of high temperature stress at squaring, flowering and boll formation stages and increased seed cotton yield and quality of cotton crop.

**Key words:** Cell injury, cotton, fiber quality, high-temperature stress, macro- and micronutrients, yield

1. Introduction

Protecting the crop from rising temperatures, particularly from extreme heat stress, has been a major concern for scientists in current and future climate scenarios (Zheng et al., 2012). Globally, air temperature is projected to rise by 2.6–4.8 °C from 2016 to 2035 (IPCC, 2014), while the temperature increasing rate during 2000–2010 has been recorded 2.2% higher than the temperature rise rate during 1970–2000. This increase in temperature due to climate change negatively affects the yield and quality of field crops (Falconnier et al., 2020). Greater efforts are expected from researchers to assess the projected impact of rising temperatures on crop yields under such expected temperature increases.

Cotton is a multipurpose cash crop; its fiber is used in textile industry and seed in vegetable oil industry. The cottonseed oil fulfils the vegetable oil demands by 18.8% in Pakistan. Pakistan is ranked fourth among cotton-producing countries worldwide. Around 1.7 million people in Pakistan are involved in cotton cultivation (Shuli et al., 2018). In Pakistan, cotton production was 7.064 million bales and grown on an area of 2.079 million hectares during 2021. Cotton contributes 0.6% to GDP and 3.1% of value added in agriculture and is the backbone

\(^{*}\) Correspondence: dr.basharat@kfueit.pk, saleemhamza312@webmail.hzau.edu.cn, shafaqataligill@yahoo.com

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in country economy (Government of Pakistan, 2021). Among all the abiotic factors that are responsible for yield reduction, high temperature is the major one (Lopes et al., 2021). The favorable temperature for cotton growth has been observed between 20 and 30 °C (Reddy et al., 1991). High-temperature stress negatively affects all life cycle stages of cotton, but the reproductive stage is the most sensitive (Snider et al., 2009). At the earlier stages, high-temperature stress reduces seed germination, seedlings, and root development. At vegetative and reproduction stages, heat stress increases the rate of transpiration and water loss, reduces photosynthetic efficiency, causes oxidative stress, and reduces seed yield and fiber quality (Reddy et al., 1997; Snider et al., 2009).

Though cotton crop favors warm environment and is often grown in hot semiarid climates, it may experience high-temperature episodes leading to yield losses (Raza and Ahmad, 2015). For example, cotton-growing areas in Pakistan often experience very high temperatures (> 45 °C) during the cotton growth season (Sarwar et al., 2017). In an earlier study, cotton lint yield was reduced by 110 kg ha⁻¹ for each 1 °C rise in maximum day temperature (Singh et al., 2007). High-temperature influences cotton production in various ways; sowing dates, plant growth and development, fiber quality, plant metabolism, biochemistry, and water relations (Singh et al., 2007). Crop physiology and biochemistry in cotton are affected by reactive oxygen species (ROS) under high temperatures (Mishra et al., 2008). High temperature also disturbs the equilibrium between ROS and the plant defense system, creating the oxidative burst that affects biomolecules and the cellular redox homeostasis (Sachdev et al., 2021). However, plants metabolize the ROS through their defense system, i.e. via the production of a set of enzymatic and nonenzymatic antioxidant systems that could be measured by assessing total soluble proteins (Keles et al., 2004; Hänisch and Mendel, 2009). Similarly, oxidative stress under high-temperature increases leaf cell membrane leakage (Sarwar et al., 2018) that reduces the hydration status of leaves (Carmo-Silva and Salvucci, 2012). Reduced physiological and biochemical activities at high temperatures (31 °C) declined fiber fineness and length (Conaty et al., 2015).

It has been reported that the application of S also acts as an osmoprotectants (Manzoor et al., 2016). The deficiency of K, Zn, and B affects the plant protection mechanisms (Koshiba et al., 2008; Demidchik et al., 2010; Peck and McDonald, 2010). However, exogenous application of these nutrients protects the membranes from oxidative stress (Cakmak, 2000; Wang et al., 2009; Hajiboland and Farhanghi, 2010). Previous reports have also demonstrated that K, Zn and B maintain potential osmoregulation, osmotic and turgor maintenance, while deficiencies of these nutrients affect plant water relations (Mouline et al., 2002; Khan et al., 2004; Stavrianakou et al., 2006). Furthermore, an increase in cotton crop yield and fiber quality have been observed with the external application of K, Zn and B under field conditions (Kim et al., 2008; Rashidi and Seilsepour, 2011; Waraich et al., 2011). However, the effect of combined application of these nutrients has not previously been quantified under different glasshouse and field thermal regimes.

Several approaches have been reported to mitigate the heat stress, such 1) development of heat tolerant genotypes (Mondal et al., 2016), 2) cultural practices i.e. changing sowing dates (Saeed et al., 2017), exogenous application of compatible solutes (Siddique et al., 2018), signaling molecules, plant growth regulators (Hu et al., 2016) and foliar spray of nutrients (Waraich et al., 2012). Foliar application of nutrients is one of the important cultural practice for mitigating the adverse effects of high temperature stress (Ragunath et al., 2021). High temperature decreases nutrient uptake, their utilization and partitioning (Mathias et al., 2021). However, the foliar application of macro/micronutrients activates the plant defense system and reinforces physiological activities (Dordas and Brown, 2005; Ahmad and Prasad, 2011). The improvement of the high-temperature stress tolerance with the exogenous application of mineral nutrients makes it an easy and economically feasible approach to alleviate that stress (Waraich et al., 2015; Seth et al., 2018). For example, K is an essential nutrient that regulates several physiological and biochemical processes of plants to reduce the oxidative stress, and has a vital role under various environmental stresses (Hossain et al., 2020). To the best of our knowledge, no work has been conducted under combined glass house and field conditions to check the comparative effects of medium and high temperature stresses at squaring, flowering and boll formation stages of cotton and also no study has been conducted to check the comparative effects of K, Zn and B for alleviating the adverse effects of high temperature stress at three reproductive stages of cotton crop.

Keeping in view the importance of heat stress at reproductive stages of cotton crop and the mitigatory role of K, Zn and B under high temperature stress, we hypothesized that the adverse effects of high temperature stress at three reproductive stages of cotton could be minimized through foliar application of macro- and micronutrients. Therefore, the present study aimed to assess the impacts of temperatures, under both glasshouse and field conditions, on cotton leaf biochemistry, water relationships, membrane stability, yield, and fiber quality traits. The study also aimed to compare the role of K, Zn, and B in mitigating the adverse effects of high-temperature stress.
2. Materials and methods

2.1.Conditions for glasshouse experiment
Cotton plants were grown in pots organized in a completely random block design with split plot arrangement in a glasshouse at the University of Agriculture Faisalabad, Pakistan. Sun was the sole source of active photosynthetic radiation (1400–1600 mmol m⁻² s⁻¹) in glass house and fluorescent bulbs were used in growth chambers to provide supplementary light, while light in glass house was set to be at 14/10 h day/night light period. The effect of temperature regimes was studied with a medium heat-tolerant cotton variety (AA-802—the variety was screened under a preliminary experiment). Soil conditions and properties were similar as described in Sarwar et al. (2017). Three temperature regimes were applied i.e. 32/20 °C (optimal: OpTR), 38/24 °C (critical point for cotton growth: SubTR), and 45/30 °C (prevails in most of the cotton-growing areas in Pakistan: SupTR) at squaring, flowering and boll formation stages of cotton. Plants were raised first at OpTR and then moved to SubTR and SupTR. A day before shifting pots in SubTR and SupTR nutrients spray i.e. water spray (control), K-1.5%, Zn-0.2% and B-0.1% were applied at squaring, flowering and boll formation stages. The same nutrients were also applied under OpTR and nutrients combinations such as K+Zn and K+Zn+B were not used. Four replications were used in completely randomized design with split arrangement. Each replication contained four pots under each thermal regime. Total soluble proteins (TSP), hydrogen peroxide (H₂O₂), relative cell injuries (RCI) and relative water contents (RWC) were measured 7 days following heat treatment. The pots were then moved back to optimal temperature regime. The experiments were completed in 120 days from the sowing date.

2.2. Conditions for field experiment
Cotton plants were also grown on the farm at Department of Agronomy, University of Agriculture Faisalabad, Pakistan and three sowing dates were used to experience different temperatures during three reproductive stages of cotton crop. For example, April, May and June sowing dates provided different temperatures to cotton crop at three reproductive stages (squaring, flowering and boll formation). April (early sowing) and May sowing dates provided high temperature at three reproductive stages of cotton crop while June (late) sowing provided optimum temperature at three reproductive stages of cotton crop and is considered as control. Foliar spray of potassium (K) (1.5%), zinc (Zn) (0.2%) and boron (0.1%) was applied at three reproductive stages of cotton one day before the onset of high temperature stress (through weather forecast). Leaf samples were collected seven days after spraying for different physiological and biochemical attributes. Each sowing date (thermal regime) and nutrients spray was replicated thrice in field conditions. The net plot size was 6.0 m × 4.5 m. A total of 12 plots (12 treatments) were used in each replication and there were 180 plants in each plot. Randomized complete block design with split plot arrangement was used to manage the layout by having sowing dates in main plots and nutrients in subplots. The climatic data were collected from the Observatory of Department of Agronomy, University of Agriculture Faisalabad, Pakistan. The tested sowing dates were April 2 and 4, May 3 and 2, June 17 and 19 during the first and second year, respectively. These sowing dates considered as thermal regimes provided different temperatures at three reproductive stages, i.e. squaring, flowering, and boll formation, and April and May thermal regimes provided high temperatures at squaring, flowering, and boll forming stages, while June thermal regime provided optimum thermal regimes for all three reproductive stages (Sarwar et al., 2017).

2.3. Chemical analysis

2.3.1. Total soluble proteins
Fully extended young leaves, mostly 4th from the top, were collected seven days after treatment application. Total soluble proteins were calculated (as mg g⁻¹ of fresh weight, FW), by quantifying protein contents through combining a total volume of 100 μL enzyme sample with 5 mL Bradford reagent. Mixture absorbance was estimated at 595 nm (Bradford, 1976).

2.3.2. Hydrogen peroxide
H₂O₂ (μ mol g⁻¹ FW) was measured following a procedure outlined in Velikova et al. (2000). The reaction mixture, i.e. 1 mL of potassium iodide and 0.5 mL of phosphate buffer (pH 7.0) was transferred in 0.5 mL hydrogen peroxide extract. The absorbance was measured at a wavelength of 390 nm.

2.3.3. Leaf relative water contents
Fresh leaf samples (0.5 g FW) were soaked overnight in distilled water to make them completely turgid, and the turgid weight (TW) of the soaked leaves was taken. The leaves were then oven-dried at 80 °C for 24 h until a constant dry weight (DW). Weatherley (1950) procedure was used to calculate the RWC:

Equation number 1: \[ \text{RWC} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100. \]

2.3.4. Relative cell injury/cell membrane thermostability
Two 10-mm diameter disks were taken from both sides of fully grown leaves. After washing them 3–4 times with double distilled water, samples were poured into test tubes containing deionized water. One set of tubes was heated in water at 50 °C, while the other set was kept at 25 °C (room temperature) for an hour. The initial electrical conductivity was measured in the test tubes with an electrical conductivity meter (Model, Jenway 4510, Japan).
3. Results

3.1. Glass house experiment

In this study, TSP, \( \text{H}_2\text{O}_2 \), and RCI increased significantly \((p < 0.01)\) under SubTR and SupTR (Table 1). Plants exposed to SubTR and SupTR showed 45% and 22% higher TSP contents (averaged across three reproductive stages), respectively than plants grown under OpTR; while, \( \text{H}_2\text{O}_2 \) and RCI increased by 71% and 73% under SubTR and SupTR plants over the plants of OpTR (Table 1). TNBPP, NSBPP, fiber fineness, fiber length, and fiber strength varied significantly \((p < 0.01)\) across the thermal regimes (Tables 1 and 2), while TNBPP in water-treated plants of SupTR and SubTR were decreased by 42% and 19%, respectively over the plants of OpTR. Similarly, fiber fineness was reduced by 16% and 29% in water-treated plants of SubTR and SupTR, respectively, over the water-treated plants of OpTR and NSBPP.

Foliar application of nutrients (K and Zn) increased TSP but decreased \( \text{H}_2\text{O}_2 \) and RCI \((p < 0.01)\) in all thermal regimes. For example, under SupTR and SubTR, K and Zn improved TSP by 1.22 folds and 1.02 folds over the water-treated leaves of respective thermal regimes. Similarly, \( \text{H}_2\text{O}_2 \) contents were decreased by 64% and 30% by K and

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Table 1. Effect of different thermal regimes and nutrients’ spray on total soluble proteins (TSP), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), relative water contents (RWC), relative cell injury (RCI) (averaged across of squaring, flowering, and boll formation stages) of cotton leaves; the total number of bolls per plant (TNBPP) and the number of sympodial branches per plant (NSBPP) under glasshouse conditions.

<table>
<thead>
<tr>
<th>Thermal regimes</th>
<th>Nutrients</th>
<th>TSP</th>
<th>( \text{H}_2\text{O}_2 )</th>
<th>RWC%</th>
<th>RCI%</th>
<th>TNBPP</th>
<th>NSBPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>4.87 ± 0.22</td>
<td>0.77 ± 0.03</td>
<td>87.21 ± 3.9</td>
<td>47.50 ± 2.1</td>
<td>47.86 ± 1.8</td>
<td>21.88 ± 0.8</td>
</tr>
<tr>
<td>32/20 °C</td>
<td>Potassium (1.5%)</td>
<td>8.68 ± 0.31</td>
<td>0.63 ± 0.02</td>
<td>88.87 ± 4.1</td>
<td>40.50 ± 1.7</td>
<td>46.55 ± 1.9</td>
<td>22.39 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>7.68 ± 0.44</td>
<td>0.58 ± 0.09</td>
<td>89.45 ± 4.2</td>
<td>39.00 ± 1.8</td>
<td>46.39 ± 2.0</td>
<td>22.31 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>6.57 ± 0.24</td>
<td>0.59 ± 0.02</td>
<td>89.29 ± 4.0</td>
<td>38.37 ± 2.0</td>
<td>46.20 ± 2.0</td>
<td>22.46 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>7.09 ± 0.21</td>
<td>1.32 ± 0.04</td>
<td>54.23 ± 2.1</td>
<td>80.00 ± 3.9</td>
<td>46.39 ± 2.0</td>
<td>22.31 ± 0.9</td>
</tr>
<tr>
<td>45/30 °C</td>
<td>Potassium (1.5%)</td>
<td>15.78 ± 0.62</td>
<td>0.80 ± 0.03</td>
<td>74.39 ± 3.0</td>
<td>57.85 ± 2.9</td>
<td>39.23 ± 1.9</td>
<td>18.89 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>16.20 ± 0.74</td>
<td>0.79 ± 0.02</td>
<td>73.06 ± 2.9</td>
<td>59.00 ± 3.0</td>
<td>39.07 ± 1.9</td>
<td>18.93 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>10.37 ± 0.41</td>
<td>0.85 ± 0.03</td>
<td>63.84 ± 2.5</td>
<td>62.28 ± 2.7</td>
<td>38.62 ± 1.7</td>
<td>18.01 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5.80 ± 0.19</td>
<td>0.83 ± 0.04</td>
<td>71.97 ± 3.2</td>
<td>62.73 ± 2.4</td>
<td>39.03 ± 1.6</td>
<td>18.78 ± 0.8</td>
</tr>
<tr>
<td>38/24 °C</td>
<td>Potassium (1.5%)</td>
<td>12.10 ± 0.48</td>
<td>0.64 ± 0.03</td>
<td>84.50 ± 3.7</td>
<td>50.73 ± 1.8</td>
<td>44.03 ± 1.8</td>
<td>20.29 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>13.85 ± 0.52</td>
<td>0.55 ± 0.02</td>
<td>84.66 ± 3.5</td>
<td>51.23 ± 1.9</td>
<td>44.06 ± 1.9</td>
<td>20.59 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>9.91 ± 0.34</td>
<td>0.70 ± 0.04</td>
<td>82.54 ± 2.8</td>
<td>49.28 ± 2.0</td>
<td>44.36 ± 1.8</td>
<td>19.92 ± 0.8</td>
</tr>
</tbody>
</table>

HSD 0.623 0.041 4.345 3.770 2.301 1.082

Values are the means of three replications \((n = 3)\) ± SE, and variables possessing the same letters are not statistically significant at \(p < 0.01\). Main factors and interaction are significant at \(p < 0.01\). Lettering is done separately for each thermal regime using the HSD of the interaction between thermal regimes and nutrients’ spray.

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After autoclaving, the samples (Model, HAU-85, Hirayam instruments, Japan) at 0.1 MPa pressure for 10 min, final EC was measured and RCI (%) was calculated according to Sullivan (1972) as follows.

Equation number 2: \[ \text{RCI} = 1 - \frac{1 - (\frac{T_1}{T_2})}{1 - (\frac{C_1}{C_2})} \times 100, \]

where \( T_1 \) and \( T_2 \) are the initial and final EC readings of heat-treated vials, while \( C_1 \) and \( C_2 \) are the initial and final EC of 25 °C vials. The CMT was then calculated by subtracting the values of RCI from 100.

2.4. Total number of bolls and number of sympodial branches per plant

The total number of bolls per plant (TNBPP) and the number of sympodial branches per plant (NSBPP) were determined and averaged from randomly selected 10 plants from each plot.

2.5. Fiber quality

For conditioning purposes, 10 g of lint sample was kept at 20 °C with 65%–68% relative humidity (6–8 h) from twenty randomized selected plants of each experimental unit. The HVI instrument was used to record the fiber fineness (µg/inch), length (mm), and strength (g/tex).

2.6. Statistical analysis

Statistix 10.1 program was used for the analysis of variance while treatments’ means were compared using honestly significant difference (HSD) test at 5% and 1% probability level under field and glasshouse conditions, respectively (Steel et al., 1997). Graphs were made by using Microsoft Excel Program.
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Table 2. Effect of different thermal regimes and nutrients’ spray-on fiber fineness (µg/inch), fiber length (mm), and fiber strength (g/tex) under glasshouse conditions.

<table>
<thead>
<tr>
<th>Thermal regimes</th>
<th>Nutrients</th>
<th>Fiber fineness</th>
<th>Fiber length</th>
<th>Fiber strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>4.13 a ± 0.20</td>
<td>26.74 a ± 1.3</td>
<td>30.81 a ± 1.4</td>
</tr>
<tr>
<td>32/20 °C</td>
<td>Potassium (1.5%)</td>
<td>4.12 a ± 0.19</td>
<td>26.52 a ± 1.2</td>
<td>30.68 a ± 1.3</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>4.10 a ± 0.17</td>
<td>26.55 a ± 1.1</td>
<td>30.53 a ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>4.15 a ± 0.16</td>
<td>26.65 a ± 1.2</td>
<td>30.60 a ± 1.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3.20 c ± 0.12</td>
<td>19.57 d ± 0.20</td>
<td>23.84 c ± 1.9</td>
</tr>
<tr>
<td>45/30 °C</td>
<td>Potassium (1.5%)</td>
<td>3.73 a ± 0.15</td>
<td>24.22 b ± 1.1</td>
<td>28.83 a ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>3.80 a ± 0.17</td>
<td>25.14 a ± 1.2</td>
<td>28.26 a ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>3.65 ab ± 0.14</td>
<td>22.13 c ± 1.0</td>
<td>26.10 b ± 2.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>3.54 b ± 0.13</td>
<td>23.95 c ± 0.20</td>
<td>25.99 b ± 1.8</td>
</tr>
<tr>
<td>38/24 °C</td>
<td>Potassium (1.5%)</td>
<td>3.93 a ± 0.18</td>
<td>24.10 a ± 1.2</td>
<td>29.20 a ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>3.94 a ± 0.18</td>
<td>24.87 a ± 1.2</td>
<td>29.35 a ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>3.91 ab ± 0.17</td>
<td>25.78 b ± 1.1</td>
<td>28.46 a ± 1.9</td>
</tr>
<tr>
<td>HSD</td>
<td></td>
<td>0.146</td>
<td>0.874</td>
<td>1.350</td>
</tr>
</tbody>
</table>

Values are the means of three replications (n = 3) ± SE, and variants possessing the same letters are not statistically significant at p < 0.01. Main factors and interaction are significant at p < 0.01. Lettering is done separately for each thermal regime using the HSD of the interaction between thermal regimes and nutrients’ spray.

Zn, respectively, under SupTR and SubTR, while RCI were decreased by 37% and 24%. Thermal regimes and nutrient spray had a major effect (p < 0.01) on RWC, TNBPP, NSBPP, fiber fineness, fiber length and fiber strength (Tables 1 and 2). For instance, RWC reduced by 61% and 23% in SupTR and SubTR, respectively, in the absence of the nutrient application. Foliar application of K and Zn increased RWC, TNBPP, NSBPP, fiber fineness, fiber length, and fiber strength under SupTR and SubTR thermal regimes, but the effect was more pronounced for SupTR. Likewise, foliar application of K and Zn increased TNBPP by 19% and 14% under SupTR and SubTR over the water-treated plants of respective thermal regimes. Similarly, K and Zn-treated leaves of SupTR and SubTR treatments increased fiber fineness by 16% and 11%, respectively, over the water-treated plants of respective thermal regimes (Tables 1 and 2).

3.1.1. Association of leaf RCI with TSP, RWC, fiber quality and yield components

The relationships between RCI with RWC, TNBPP, and fiber quality (fiber fineness, fiber length and fiber strength) were assessed with regression analyses, and the effects of different nutrients were investigated under different thermal regimes (Figures 1a–1f). The relationship was substantially different for three thermal regimes, although RCI had a poor negative relation with TSP, RWC, yield, and fiber quality under OpTR, but RCI showed significant strong negative associations in SubTR (p < 0.05) and SupTR (p < 0.01). The R-squared values indicated that 4.3%–12.8% of variance in the variance of TSP, RWC, fiber quality, and yield components could be explained by RCI at the OpTR, but RCI could explain 47%–55% of the variance of the same variables at the SubTR, and 87%–96% of the variance at the SupTR (Figure 1).

3.1.2. Association of leaf TSP with RWC, yield and fiber quality components, and association of RCI with H₂O₂

The relationships between TSP with RWC, TNBPP, quality parameters (fiber fineness, fiber length, and fiber strength; Figures 2a–2e) and RCI with H₂O₂ (Figure 2f) were analyzed under various thermal regimes, while the strength of the relationship was significantly differed across the thermal regimes. Regardless of the degree of relationship, leaf TSP had insignificant positive correlations with RWC, TNBPP, and fiber quality under OpTR. The associations of TSP with RWC, TNBPP, and quality parameters were positive and significantly strong in SubTR (p < 0.05) and SupTR treatments (p < 0.01), whereas RCI was substantially and positively linked with H₂O₂ in SubTR (p < 0.05) and SupTR (p < 0.01; Figure 2f). The R-squared values indicated that 14%–21% of the variance in the variance of TSP, RWC, fiber quality, and yield components could be explained by TSP at OpTR, but TSP could explain 55%–60% of the variance of the same variables at SubTR, and 87%–96% of the variance at the SupTR (Figure 2).
3.2. Field experiment

Leaf TSP, H$_2$O$_2$ contents, and RCI were significantly higher ($p < 0.05$) in crops planted in April and May (high-temperature regimes) over the June thermal regime, for instance, TSP was increased by 55% and 29% in water-treated plants under SupTR and SubTR of April and May thermal regimes (averaged over three reproductive stages and two years of study) compared with water control plants of June thermal regime (Table 3). Likewise, H$_2$O$_2$ was increased by 68% and 37% and RCI was increased by 48% and 19% under supra- and subthermal regimes of April and May thermal regimes, respectively, over the plants of June thermal regime. Different thermal regimes varied significantly in RWC, fiber fineness, fiber length,
and fiber strength ($p < 0.05$; Tables 3–5), for example, the plants of April and May thermal regimes produced lower RWC, fiber fineness, fiber length and fiber strength than plants of June thermal regime. The RWC reduced by 57% in water control plants of May thermal regime over the water-treated plants of June sowing date. Likewise, TNBPP, NSBPP, fiber fineness, fiber length, and fiber strength were reduced in water-treated plants of April and May sown plants over the water-treated plants of June sown crop.

Foliar application of K and Zn increased TSP in cotton crop irrespective of reproductive stages but reduced $H_2O_2$ and RCI under all thermal regimes, with prominent...
Table 3. Effect of different thermal regimes and nutrients' spray on total soluble proteins (TSP), hydrogen peroxide (H$_2$O$_2$), relative water contents (RWC) and relative cell injury (RCI) (averaged across of squaring, flowering and boll formation stages) of cotton leaves under field conditions during 2012 and 2013.

<table>
<thead>
<tr>
<th>Thermal regimes</th>
<th>Nutrients</th>
<th>TSP 2012</th>
<th>TSP 2013</th>
<th>H$_2$O$_2$ 2012</th>
<th>H$_2$O$_2$ 2013</th>
<th>RWC (%) 2012</th>
<th>RWC (%) 2013</th>
<th>RCI (%) 2012</th>
<th>RCI (%) 2013</th>
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</thead>
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<tr>
<td><strong>Optimal regimes</strong></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>of sowing dates</td>
<td>Control</td>
<td>4.19 b ± 0.31</td>
<td>4.15 b ± 0.37</td>
<td>0.78 a ± 0.06</td>
<td>0.74 a ± 0.06</td>
<td>85.94 ± 7.6</td>
<td>84.47 ± 7.8</td>
<td>45.07 ± 4.2</td>
<td>48.99 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>Potassium (1.5%)</td>
<td>7.01 a ± 0.74</td>
<td>7.21 a ± 0.72</td>
<td>0.61 ab ± 0.04</td>
<td>0.61 ab ± 0.04</td>
<td>85.89 ± 7.8</td>
<td>84.42 ± 7.3</td>
<td>36.98 b ± 3.3</td>
<td>40.56 b ± 3.6</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>7.30 a ± 0.89</td>
<td>7.58 a ± 0.97</td>
<td>0.63 ab ± 0.03</td>
<td>0.61 ab ± 0.04</td>
<td>88.66 ± 8.2</td>
<td>87.19 ± 8.1</td>
<td>36.80 b ± 3.8</td>
<td>41.22 b ± 3.2</td>
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<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>6.20 a ± 0.64</td>
<td>6.43 a ± 0.80</td>
<td>0.69 a ± 0.05</td>
<td>0.66 a ± 0.05</td>
<td>89.61 ± 8.5</td>
<td>88.14 ± 8.4</td>
<td>37.07 b ± 3.6</td>
<td>41.22 b ± 2.9</td>
</tr>
<tr>
<td><strong>Supraoptimal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>regime of sowing dates</td>
<td>Control</td>
<td>6.70 d ± 0.45</td>
<td>6.10 d ± 0.41</td>
<td>1.33 a ± 0.09</td>
<td>1.21 a ± 0.11</td>
<td>57.45 ± 4.9</td>
<td>57.98 ± 4.7</td>
<td>80.90 ± 7.4</td>
<td>73.45 ± 6.8</td>
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<td>Potassium (1.5%)</td>
<td>14.95 b ± 1.22</td>
<td>13.83 b ± 1.12</td>
<td>0.89 c ± 0.06</td>
<td>0.76 b ± 0.06</td>
<td>76.70 ± 6.9</td>
<td>78.24 ± 6.4</td>
<td>56.30 b ± 5.4</td>
<td>49.85 b ± 4.2</td>
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<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>17.11 a ± 1.46</td>
<td>16.03 a ± 1.40</td>
<td>0.92 c ± 0.05</td>
<td>0.79 b ± 0.05</td>
<td>77.37 ± 7.1</td>
<td>78.90 ± 6.3</td>
<td>55.23 b ± 5.8</td>
<td>50.79 b ± 4.7</td>
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<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>10.30 c ± 0.74</td>
<td>10.85 c ± 0.95</td>
<td>1.01 b ± 0.07</td>
<td>0.92 b ± 0.07</td>
<td>66.67 ± 5.9</td>
<td>68.21 ± 5.7</td>
<td>61.83 b ± 4.9</td>
<td>55.45 b ± 4.8</td>
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<tr>
<td><strong>Suboptimal</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>regime of sowing dates</td>
<td>Control</td>
<td>5.40 c ± 0.35</td>
<td>5.60 d ± 0.39</td>
<td>1.07 a ± 0.07</td>
<td>1.01 a ± 0.07</td>
<td>79.45 ± 7.0</td>
<td>82.94 ± 7.2</td>
<td>65.95 a ± 4.5</td>
<td>66.75 a ± 5.1</td>
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<td></td>
<td>Potassium (1.5%)</td>
<td>11.80 a ± 0.92</td>
<td>11.23 b ± 0.97</td>
<td>0.82 b ± 0.04</td>
<td>0.78 b ± 0.04</td>
<td>80.70 ± 7.6</td>
<td>82.89 ± 7.6</td>
<td>53.36 b ± 3.8</td>
<td>57.08 b ± 4.1</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>13.33 a ± 1.14</td>
<td>12.90 a ± 1.03</td>
<td>0.84 b ± 0.04</td>
<td>0.80 b ± 0.03</td>
<td>83.37 ± 7.2</td>
<td>85.66 ± 7.9</td>
<td>55.95 b ± 4.1</td>
<td>56.75 b ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>9.90 b ± 0.71</td>
<td>9.31 ± 0.78</td>
<td>0.93 ± 0.05</td>
<td>0.89 b ± 0.06</td>
<td>79.67 ± 6.5</td>
<td>86.61 ± 8.1</td>
<td>57.28 b ± 3.9</td>
<td>59.10 b ± 4.5</td>
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<tr>
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<td>HSD</td>
<td>1.891</td>
<td>1.645</td>
<td>0.134</td>
<td>0.127</td>
<td>8.156</td>
<td>9.680</td>
<td>7.093</td>
<td>6.403</td>
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</table>

Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at $p < 0.05$. Lettering is done separately for each thermal regime using the HSD of the interaction between thermal regimes and nutrients' spray.
Table 4. Effect of different thermal regimes and nutrients’ spray on the total number of bolls per plant (TNBPP), number of sympodial branches per plant (NSBPP), fiber fineness (µg/inch), and fiber length (mm) of cotton crop under field conditions during 2012 and 2013.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>April (High temperature)</td>
<td>Control</td>
<td>45.34 b ± 3.8</td>
<td>38.91 b ± 3.0</td>
<td>21.87 b ± 1.9</td>
<td>20.75 b ± 1.8</td>
<td>4.02 b ± 0.38</td>
<td>3.28 b ± 0.28</td>
<td>21.00 b ± 1.8</td>
<td>21.15 b ± 1.9</td>
</tr>
<tr>
<td></td>
<td>K (1.5%)</td>
<td>50.64 a ± 4.5</td>
<td>43.85 a ± 3.6</td>
<td>25.95 a ± 2.1</td>
<td>24.69 a ± 2.1</td>
<td>4.72 a ± 0.42</td>
<td>3.98 a ± 0.33</td>
<td>25.07 a ± 2.2</td>
<td>24.69 a ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Zn (0.2%)</td>
<td>51.55 a ± 4.3</td>
<td>43.09 a ± 3.5</td>
<td>25.99 a ± 2.1</td>
<td>24.80 a ± 2.1</td>
<td>4.69 a ± 0.41</td>
<td>4.10 a ± 0.40</td>
<td>25.65 a ± 2.2</td>
<td>25.53 a ± 2.2</td>
</tr>
<tr>
<td></td>
<td>B (0.1%)</td>
<td>49.32 a ± 4.3</td>
<td>43.27 a ± 3.5</td>
<td>24.91 a ± 2.0</td>
<td>24.47 a ± 2.0</td>
<td>4.82 a ± 0.43</td>
<td>3.96 a ± 0.34</td>
<td>25.88 a ± 2.3</td>
<td>23.50 a ± 2.0</td>
</tr>
<tr>
<td>May (High temperature)</td>
<td>Control</td>
<td>36.15 b ± 3.1</td>
<td>38.34 b ± 3.1</td>
<td>20.62 b ± 1.7</td>
<td>19.92 b ± 1.5</td>
<td>3.20 b ± 2.90</td>
<td>3.53 b ± 1.8</td>
<td>19.53 c ± 1.7</td>
<td>20.67 b ± 1.8</td>
</tr>
<tr>
<td></td>
<td>K (1.5%)</td>
<td>42.51 a ± 3.9</td>
<td>45.05 a ± 4.1</td>
<td>23.69 a ± 1.9</td>
<td>23.92 a ± 1.8</td>
<td>3.90 a ± 0.38</td>
<td>4.30 a ± 0.37</td>
<td>23.76 a ± 2.1</td>
<td>24.74 a ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Zn (0.2%)</td>
<td>41.08 a ± 3.8</td>
<td>43.62 a ± 3.9</td>
<td>23.67 a ± 1.9</td>
<td>25.27 a ± 2.1</td>
<td>4.28 a ± 0.41</td>
<td>4.39 a ± 0.39</td>
<td>24.59 a ± 2.2</td>
<td>24.32 a ± 2.1</td>
</tr>
<tr>
<td></td>
<td>B (0.1%)</td>
<td>40.26 a ± 3.7</td>
<td>42.80 a ± 3.8</td>
<td>23.57 a ± 1.8</td>
<td>24.67 a ± 1.9</td>
<td>3.95 a ± 0.38</td>
<td>4.37 a ± 0.38</td>
<td>22.89 ab ± 1.9</td>
<td>25.55 a ± 2.2</td>
</tr>
<tr>
<td>June (late sown as optimal temperature)</td>
<td>Control</td>
<td>35.11 a ± 2.9</td>
<td>36.45 a ± 2.8</td>
<td>21.59 a ± 1.8</td>
<td>21.89 a ± 1.8</td>
<td>4.42 a ± 0.37</td>
<td>4.52 a ± 0.42</td>
<td>27.15 a ± 2.4</td>
<td>26.98 a ± 2.3</td>
</tr>
<tr>
<td></td>
<td>K (1.5%)</td>
<td>37.11 a ± 3.0</td>
<td>36.45 a ± 2.7</td>
<td>21.11 a ± 1.7</td>
<td>21.41 a ± 1.7</td>
<td>4.43 a ± 0.38</td>
<td>4.48 a ± 0.41</td>
<td>27.24 a ± 2.4</td>
<td>27.07 a ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Zn (0.2%)</td>
<td>37.34 a ± 3.1</td>
<td>37.68 a ± 2.9</td>
<td>21.69 a ± 1.8</td>
<td>21.99 a ± 1.9</td>
<td>4.35 a ± 0.39</td>
<td>4.45 a ± 0.40</td>
<td>26.82 a ± 2.3</td>
<td>26.65 a ± 2.5</td>
</tr>
<tr>
<td></td>
<td>B (0.1%)</td>
<td>37.4 a ± 3.2</td>
<td>37.08 a ± 2.9</td>
<td>21.15 a ± 1.7</td>
<td>21.45 a ± 1.7</td>
<td>4.37 a ± 0.40</td>
<td>4.29 a ± 0.39</td>
<td>27.93 a ± 2.5</td>
<td>27.76 a ± 2.6</td>
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<tr>
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<td>HSD</td>
<td>3.946</td>
<td>3.987</td>
<td>2.627</td>
<td>3.571</td>
<td>0.621</td>
<td>0.614</td>
<td>2.102</td>
<td>2.342</td>
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</table>

Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at $p < 0.05$. Lettering is done separately for each thermal regime using the HSD of the interaction between thermal regimes and nutrients’ spray.
response under the high-temperature regimes (April and May sowing; Table 3). Both K and Zn-treated leaves followed by B (averaged across) had 1.58-fold, 1.20-fold, and 0.68-fold higher TSP under supraoptimal, suboptimal, and optimal thermal regimes than water-treated leaves of respective thermal regimes, while both K and Zn (averaged across) decreased $H_2O_2$ contents by 52% and 29% over control plants under supra- and subthermal regimes (averaged over three reproductive stages and two years of study). Application of K and Zn decreased RCI by 48% and 19% under supra- and subthermal regimes (April and May sown crops), respectively, than their respective control plants (averaged across three reproductive stages and both years of study). Further, RWC, TNBPP, NSBPP, fiber fineness, fiber length and fiber strength were also improved by K and Zn followed by B under all thermal regimes ($p < 0.05$), but the impact was more prominent under April and May sown crops (Tables 3–5). Application of K and Zn (averaged over three reproductive stages and two years of study) improved RWC by 36% under the high-temperature regime (May) than water-treated plants under the respective thermal regime. Application of K and Zn (averaged over both years of study) increased the total number of bolls by 14% and 16% under April and May thermal regimes than that of control plants of respective sowing dates; while, foliar spray of K and Zn (averaged across) increased fiber fineness by 26% and 23% under April and May thermal regimes than the water-treated plants of respective thermal regimes. Likewise, under April and May sowing dates, application of K and Zn improved NS/ plant, fiber length, and fiber strength.

3.2.1. Association of leaf RCI with TSP, RWC, fiber quality and yield components

The relationships between RCI with RWC, TNBPP, and quality parameters were evaluated by regression analysis to test the impact of different nutrients under various thermal regimes (Figures 3a–3f), and April and May thermal regimes showed strong negative correlations ($p < 0.05, p < 0.01$) of RCI to all other parameters, while RCI showed insignificant negative associations with other parameters under June (control) thermal regime. Whereas $R^2$ showed that between 52% and 91% of the variance of TSP, RWC, fiber quality, and yield components could be explained by RCI in April and May thermal regimes, only 3%–15% of the variance in the same variables explained by RCI in June thermal regime (Figure 3).

3.2.2. Association of leaf TSP with RWC, fiber quality and yield components; and association of RCI with $H_2O_2$

Similar to the findings from glass house experiment, TSP showed a low but positive association with RWC, total number of bolls and fiber quality parameters under optimal temperature regimes (Figures 4a–4e), while April and May sown crops demonstrated strong positive relationships of TSP with RWC, TNBPP, fiber fineness, fiber length, and fiber strength (Figure 4e). The mean squares of regression were highly significant ($p < 0.01$) under April and May

<table>
<thead>
<tr>
<th>Thermal regimes</th>
<th>Nutrients</th>
<th>Fiber strength 2012</th>
<th>Fiber strength 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>April (High temperature)</td>
<td>Control</td>
<td>27.10 b ± 2.2</td>
<td>27.52 b ± 2.4</td>
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<td></td>
<td>Potassium (1.5%)</td>
<td>30.80 a ± 2.6</td>
<td>31.61 a ± 2.8</td>
</tr>
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<td>Zinc (0.2%)</td>
<td>30.76 a ± 2.5</td>
<td>31.40 a ± 2.8</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>30.77 a ± 2.6</td>
<td>30.20 ab ± 2.6</td>
</tr>
<tr>
<td>May (High temperature)</td>
<td>Control</td>
<td>23.83 b ± 2.0</td>
<td>24.85 b ± 2.0</td>
</tr>
<tr>
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<td>Potassium (1.5%)</td>
<td>27.90 a ± 2.3</td>
<td>28.94 a ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>27.83 a ± 2.3</td>
<td>28.67 a ± 2.4</td>
</tr>
<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>27.71 a ± 2.2</td>
<td>28.62 a ± 2.3</td>
</tr>
<tr>
<td>June (late sown as optimal)</td>
<td>Control</td>
<td>29.28 a ± 1.8</td>
<td>28.50 a ± 2.3</td>
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<td></td>
<td>Potassium (1.5%)</td>
<td>30.37 a ± 2.7</td>
<td>30.50 a ± 2.6</td>
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<tr>
<td></td>
<td>Zinc (0.2%)</td>
<td>29.07 a ± 2.6</td>
<td>30.35 a ± 2.6</td>
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<tr>
<td></td>
<td>Boron (0.1%)</td>
<td>29.96 a ± 2.6</td>
<td>30.17 a ± 2.5</td>
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<td>3.804</td>
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Values are the means of three replications (n = 3) ± SE and variants possessing the same letters are not statistically significant at $p < 0.05$. Lettering is done separately for each thermal regime using the HSD of the interaction between thermal regimes and nutrients’ spray.
The relationships between relative cell injury and (a) total soluble proteins, (b) relative water contents (c) total number of bolls per plant, (d) fiber fineness, (e) fiber length and (f) fiber strength under field conditions at three thermal regimes (averaged across of three reproductive stages). * and ** indicate significance at 5% and 1% levels, respectively.

thermal regimes and the points of regression along the regression line showed strong negative associations. Regardless of the association points, leaf RCI had a positive and strong relationship to $\text{H}_2\text{O}_2$ under high-temperature regimes (Figure 4f), and R-squared values indicate that 57%–87% of the variance of RWC, fiber quality, and yield components could be explained by TSP in April and May thermal regimes. However, only 14%–39% of the variance in the same variables could be explained by TSP in June thermal regime (Figure 4).

4. Discussion
Application of adequate and balanced availability of essential macro- and micronutrients ensures healthy plants. The foliar applications of nutrients could mitigate the negative effects of abiotic stress, such as heat, and

\[ y = -0.908x + 79.06 \]
\[ R^2 = 0.682 \]
\[ t = -5.39^{**} \]
\[ SE = 0.12 \]

\[ y = -0.484x + 75.84 \]
\[ R^2 = 0.880 \]
\[ t = -7.25^{**} \]
\[ SE = 0.068 \]

\[ y = -0.180x + 53.81 \]
\[ R^2 = 0.104 \]
\[ t = -0.76 \text{ns} \]
\[ SE = 0.21 \]

\[ y = -0.518x + 35.14 \]
\[ R^2 = 0.645 \]
\[ t = -3.44^{**} \]
\[ SE = 0.13 \]

\[ y = -0.208x + 23.96 \]
\[ R^2 = 0.908 \]
\[ t = -7.99^{**} \]
\[ SE = 0.024 \]

\[ y = -0.288x + 28.68 \]
\[ R^2 = 0.153 \]
\[ t = -0.67 \text{ns} \]
\[ SE = 0.27 \]

\[ y = -0.814x + 118.3 \]
\[ R^2 = 0.772 \]
\[ t = -5.11^{**} \]
\[ SE = 0.13 \]

\[ y = -0.507x + 104.4 \]
\[ R^2 = 0.814 \]
\[ t = -5.58^{**} \]
\[ SE = 0.09 \]

\[ y = -0.208x + 99.88 \]
\[ R^2 = 0.085 \]
\[ t = -1.10 \text{ns} \]
\[ SE = 0.19 \]

\[ y = -0.108x + 8.928 \]
\[ R^2 = 0.642 \]
\[ t = -3.42^{**} \]
\[ SE = 0.027 \]

\[ y = -0.040x + 7.282 \]
\[ R^2 = 0.833 \]
\[ t = -6.11^{**} \]
\[ SE = 0.006 \]

\[ y = -0.020x + 5.766 \]
\[ R^2 = 0.106 \]
\[ t = -0.93 \text{ns} \]
\[ SE = 0.024 \]
enhance tolerance in plants (Ahmad and Prasad, 2011; Seleiman et al., 2019). High-temperature influences plant biochemistry and metabolism (Wu et al., 2016). Stress prevents crop growth and development; though reproductive processes are the most vulnerable to stressful conditions (Fahad et al., 2017). Being a cost-effective and efficient method, foliar application of nutrients could be an important way of managing environmental stresses.

Leaf total soluble proteins and hydrogen peroxide increased prominently from medium to high temperature regimes of present study over the optimal temperature regime. The increase in total soluble proteins (TSP) under medium and high temperature stress reduces the concentration of hydrogen peroxide but this increase in TSP and antioxidants are not enough to maintain a balance between ROS and plant defensive system (Sreenivasulu et al., 2019).

**Figure 4.** The relationships between total soluble proteins and (a) relative water contents, (b) total number of bolls per plant, (c) fiber fineness, (d) fiber length, (e) fiber strength and (f) relative cell injury with hydrogen peroxide under field conditions at three thermal regimes (averaged across three reproductive stages). * and ** indicate significance at 5% and 1% levels, respectively.
et al., 2007; Sarwar et al., 2019). The lower production of TSP under high temperature stress could be due to the suppression of genes and the denaturation of proteins under high temperature stress (Rollins et al., 2013; Cottee et al., 2014; Zafar et al., 2018). Previously, Chen et al. (2005) documented that high temperature stress reduces total soluble proteins and the gene expression in cotton due to oxidative stress. Foliar application of K and Zn significantly increased total soluble proteins and decreased H$_2$O$_2$ contents of present study over the water-treated and boron treated plants. Foliar application of K and Zn reduced membrane leakage due to oxidative stress (Sperry et al., 1998; Pei et al., 2000). Machado et al. (2015; Aksu and Altay, 2020; Singh et al., 2018) documented that high temperature stress reduces total soluble proteins and the gene expression in cotton crop damages the integral and peripheral proteins of cell membranes leading towards membrane leakage. Foliar spray of K and Zn reduced membrane leakage under medium and high temperature regimes of present study over the water-treated plants. The reduction in membrane leakage under high temperature stress by K and Zn could be due to the production of osmoprotectants, compatible solutes and the formation of bonds with membranes and phenolic, which protects the membranes from oxidative damage (Sarwar et al., 2019; Ju et al., 2021; Weisany et al., 2011). The foliar and soil application of K and Zn in wheat and sugar beet under heat and drought stresses reduces membrane damage due to the stability of lipid bilayer of membranes and the reduction in oxidative stress (Ghanepour et al., 2015; Aksu and Altay, 2020; Singh et al., 2020). Leaf water contents were reduced prominently under medium and high temperature stress but the more reduction was observed under high temperature over the water and boron treated plants. The reduction in leaf water contents under high temperature stress could be due to the inability of roots to uptake water and nutrients, which reduces the solute and water potential of leaves for proper leaf turgidity (Sperry et al., 1998; Pei et al., 2000). Machado and Paulsen (2001) and Wahid and Close (2007) reported that high temperature stress in sugarcane and wheat crops at initial growth stages reduces leaf water contents due to more evapotranspiration and faster depletion of soil water. Foliar application of K and Zn improved leaf water contents under high temperature stress of present study, which might be due to the ability of K and Zn to provide a favorable gradient for water and solute potential. The foliar spray of K and Zn might increase the production of compatible solutes in field crops under high temperature stress that increases the osmotic adjustment and water contents in leaves (Abdallah et al., 2019). Shahid et al. (2019) and Yavas et al. (2016) also reported that foliar spray of K and Zn under heat and drought stresses of wheat at grain filling stages improve the osmotic adjustment and leaf turgidity for higher water contents. High temperature stress at reproductive stages of cotton under glasshouse and field conditions causes substantial yield and quality reduction (Iqbal et al., 2017; Ekinci et al., 2017; Kanwal et al., 2021). For example, for each 1 °C rise in maximum daytime temperature causes a lint yield reduction of 110 kg ha$^{-1}$ (Singh et al., 2007). Similar to the findings of earlier workers, the high temperature stress of present study significantly reduced the yield attributes and fiber quality. The membrane leakage due to oxidative stress under high temperature stress of cotton could reduce the health of PS-II to PS-I, leaf water contents, which reduces yield and fiber quality attributes (Szymańska et al., 2017; Majeed et al., 2019). Demmig-Adams et al. (2018) and Zafar et al. (2021) reported that high temperature stress in cotton affects the synthesis of carbohydrates and its translocation towards yield attributes and the fiber. Under nutrient deficiency in field crops, PS-II reaction centre overactive due to reduced CO$_2$ assimilation and become the cause of oxidative stress (Singh and Reddy, 2016; Rai-Kalal et al., 2021). Foliar application of K, Zn and B increased the yield attributes and the quality of cotton fiber under high temperature stress of present study over the water-treated plants. The increase in yield attributes and fiber quality by K, Zn, and B in cotton under high temperature stress could be due to the stimulation of plant defensive system, which reduced the oxidative stress, increased membrane stability and consequently increased fiber yield and quality (Sankanararayanan et al., 2010). Exogenous application of K, Zn and B increases yield and quality of cotton under heat and drought stresses due to the increased efficiency of PS-II, which increases the production of carbohydrates and the yield attributes (Hu et al., 2016; Loka et al., 2020).

A strong positive link between total soluble proteins, yield and fiber-quality components indicates the key role of total soluble proteins in thermodurability. This study showed clear adverse relationships between membrane damage with cell proteins, water contents, yield and quality components of the cotton crop, showing that heat damage to membranes affects cotton crops' biochemistry, yield and quality components as reported previously (Rahman et
The strong positive relation between membrane damage and ROS suggests that oxidative stress is a principal cause of membrane leakage under high-temperature stress. It shows that membrane stability represents a heat tolerance measure (Cottee et al., 2007), and membrane leakage starts to rise as the temperature reaches more than 30 °C (Bibi et al., 2008).

5. Conclusion
Membrane leakage and oxidative stress due to high-temperature stress decreased cotton crop yield and fiber quality. The low yield was due to low numbers of bolls and sympodial branches associated with lower total soluble proteins and water contents. The strong negative relationships of RCI to cell biochemistry, yields, and fiber quality indicate that membrane leakage might be the main cause of low yield. Various macro- and micronutrients (K and Zn) induced thermotolerance in cotton plants by strengthening the biochemistry and membrane stability, increased yield, and fiber quality through water interactions. These nutrients could be used before the onset of high-temperature stress. Future research is needed to assess the role of these nutrients in signaling for thermotolerance under high-temperatures stress, especially in the development of heat-shock proteins by K and Zn.

Acknowledgments
The authors are grateful to the Analytical Lab, Department of Agronomy, Faisalabad University of Agriculture, Pakistan for providing technical assistance, and the Pakistan Higher Education Commission (HEC) for financial support. The authors would like to express their deepest gratitude to University of Tabuk, for the technical and financial support for this study.

Funding
The publication of the present work is supported by the Natural Science Basic Research Program of Shaanxi Province (grant no. 2018Q5218) and the National Natural Science Foundation of China (51809224), Top Young Talents of Shaanxi Special Support Program.

Conflicts of interest
The authors declare that there are no conflict of interest.

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